EXPLORING THE BEHAVIOUR OF BIOACTIVE COMPOUNDS IN VARIOUS NANOSCOPIC MEDIA

REVIEW

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Introduction

Bioactive Compounds

A bioactive compound is simply a substance that involves biological activity.[1] It is a compound which has the capability to interact with one or more components of the living tissue by presenting a wide range of probable effects. It contain chemicals that are found in small quantities in plants and certain foods (such as fruits, vegetables, nuts and whole grains); they have actions in the body that can promote good health.

Essence and Origin

The origin of these compounds can be natural: terrestrial or aquatic; a plant, animal or other source (e.g. living organism and microorganisms) or synthetic: partially or totally.

Bioactive Compounds in Plants

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in humans and animals.[2] The secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development and are not necessary for plant growth but play an important role in competition, defense, attraction and signaling.[3]

Bioactive Compounds in Food

The term "food bioactive component" refers to non-essential bio-molecules that are present in food and have the ability to modulate metabolic processes which results in the promotion of better heath.[4]

Bioactive Compounds in Animals

These compounds are found in other living organisms and microorganisms such as bacteria,[5] mushroom and in some groups of animals.[6] Example in animal products are fatty acids, found in milk and fish.

Utility of Using Bioactive Compound

Today, there is an increasing demand for natural bioactive compounds as people express more concern about their health, especially in connection with health-giving diets but there is insufficient research into the effectiveness and safety of these substances. Now a days, synthesis or extraction of bioactive compounds from naturally occurring sources have been subject of extensive research because of their widespread application in traditional medicine,[7-11] functional food industries as well as pharmaceutical industries. These type of compounds are biologically active, possessing significant antibiotic, anti-carcinogenic, anti-viral, anti-fungal, anticancer, anti-malarial, anti-oxidant, cyto-toxic and anti-inflammatory properties.[12] Bioactive compounds reacts in human body that may promote good health and effective in prevention of cancer, heart disease, and other chronic diseases. These compounds are potential source of new materials for commercial and biomedical purposes. Their potency, specificity and usefulness help to revive the physiological function by understanding the pharmacological and chemical properties.

Some common examples of bioactive compounds:

Bioactive Compounds	Examples	Sources
Flavonoids Flavonols	Quercetin, Kaempherol	Cherry tomato, onion, broccoli, tea, red wine, berries
Flavones	Apigenin, Luteolin	Cereals, parsley, celery
Flavanones	Hesperetin, Naringenin	Citrus fruits
Flavanols	Catechins	Chocolate, beans, apricot, tea, red wine, cherry, apple
Anthocyanidins	Cyanidin	Aubergine, berries, red wine, red cabbage
Isoflavones	Daidzein, Genistein	Soy products, peas

Phenolic acid Hydroxybenzoic acid	Gallic acid Stilbenes	Berries Onion
Hydroxycinnamic acid	Caffeic acid Ferulic acid	Blueberry, kiwi, cherry, plum, apple, grains
Stilbenes	Resveratrol	Wine, grapes, blueberries
Lignans	Matairesinol	Linseed, lentils, cereals, garlic
Carotenoids	Lycopene	Tomatoes, tomato products
Isothiocyanates	Sulforaphane	Broccoli, cauliflower, cabbage, etc.
Monoterpenes	d-Limonene, perillic acid	Essential oils of citrus fruit, cherries, mint, herbs

Luminescence properties of bioactive compounds

Most of the bioactive compounds are highly luminescent in nature which gives huge advantage for the study of bioactive compounds by luminescence spectroscopy. Luminescence is the emission of light from any substance and generally occurs from electronically excited states. Luminescence is formally divided into two categories, fluorescence and phosphorescence, depending on the nature of the excited states whether it is singlet or triplet respectively. In both excited and ground state for singlet state the electrons are paired. Consequently, return to the ground state is spin allowed and occurs rapidly by emission of a photon. This type of emission is called fluorescence emission. The emission rate of fluorescence is typically 10^8 per sec.

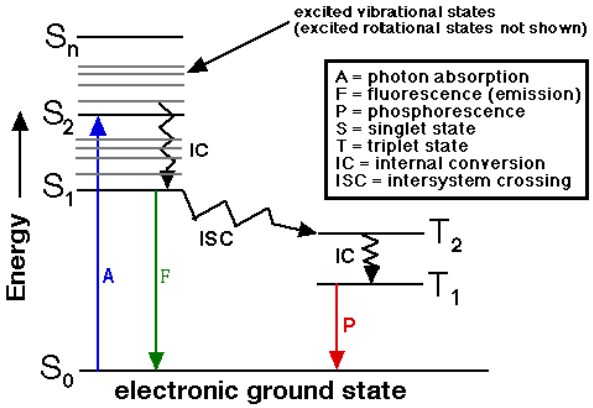

Fig. 1 Jablonski diagram showing radiative (straight lines) and non-radiative (sinuous lines) electron transition processes. The electronic ground and excited states (S_0, S_1, S_2 and T_1) show further differentiation into vibrational levels.

The processes which occur between the absorption and emission of light are usually illustrated by a **Jablonski diagram**. Upon the excitation of light a fluorophore is usually promoted to its higher electronic energy states i.e. S_2, S_3 etc. or higher vibrational level of S_1. Then it rapidly lose some of its excess energy to the surroundings as heat and return to ground vibrational level of the first excited state (S_1), which is termed as internal conversion (IC) and generally occurs in 10^{-12} s or less. Since fluorescence lifetimes are typically near 10^{-8} s, internal conversion is generally complete prior to emission. Hence fluorescence emission generally results from a thermally equilibrate exited state, that is the lowest energy vibrational state of S_1. Similar kind of energy loss may also lead to the transfer of singlet excited state to triplet excited state, this radiation less transition is called intersystem crossing (ISC). Then the molecule from the lowest vibrational level of S_1 or T_1 state return back to its ground state (S_0) either by non-radiative or radiative ways. Non-radiative transitions from S_1 or T_1 to S_0 are termed as IC and ISC, respectively. The radiative transition results luminescence emission and it is further distinguished by the term fluorescence ($S_1 \rightarrow S_0$) and phosphorescence ($T_1 \rightarrow S_0$), respectively.

Absorption, Emission and Excitation spectra:

Absorption spectrum can be obtained by scanning through the absorption band, measuring the ratio of transmitted light to incident light at each wavelength. This gives the extinction coefficient. To get an emission spectrum, a location in the absorption manifold is taken and then the power emitted at each wavelength was measured. An excitation spectrum can be measured by selecting a location in the emission manifold and scanning the excitation wavelength through the excitation manifold, then measuring the emitted power at each wavelength.

Absorption spectrum: $A(\lambda) = \epsilon(\lambda)$

Emission spectrum: $F(\lambda; \lambda_{em}) = \Phi_0 \, \epsilon(\lambda_{ex}) f(\lambda)$

Excitation spectrum: $F(\lambda; \lambda_{ex}) = \Phi_0 \, \epsilon(\lambda) f(\lambda_{em})$

Only the magnitude of the emission spectrum is affected by the choice of excitation wavelength.

Characteristics of Fluorescence Emission:

1. Fluorescence emission is Stokes shifted with respect to absorption.

Stokes Shift: The Stokes Shift is the difference, in nanometres, between the peak excitation and the peak emission wavelengths. The Stokes shift can be expressed as a function of solvent properties and the dipole moments of the fluorophore in the ground and excited states. Each fluorophore has a distinct and individual Stokes Shift. For example, in case of coumarin dyes the Stokes shift generally increases with solvent polarity (i.e., the emission maximum shifts further bathochromically than the absorption maximum).

2. Fluorescence emission obeys mirror image rule (Levschin's Rule): Since some transitions in opposite directions are involved for absorption and emission, thus fluorescence emission is generally a mirror image of ($S_0 \rightarrow S_1$).

3. Fluorescence emission remains invariant with change in excitation wavelength (Kasha's Rule): When a fluorophore is excited into higher vibrational levels, the excess energy is quickly dissipated leaving it in vibrationally relaxed singlet S_1. Due to this rapid relaxation, same fluorescence emission is generally observed irrespective of the excitation wavelength.

Fluorescence Quantum Yield: The fluorescence quantum yield φ_f of a fluorophore is the ratio of fluorescence photons emitted to photons absorbed. According to the following equation,

$$\boldsymbol{\varphi_f = \frac{k_r}{(k_r + k_{nr})}}$$

the quantum yield can be described by two rate constants, the radiative rate constant, k_r and the non-radiative rate constant, k_{nr} both depopulate the excited state. The quantum yield can be close to unity if $k_{nr} << k_r$. The yield typically depends on pH, temperature, and environment, but can be within a few % of 1. It is an intrinsic property of fluorophore.

Fluorescence Lifetime: The fluorescence lifetime is a measure of the time a fluorophore spends in the excited state before returning to the ground state by emitting a photon. The lifetimes of fluorophores can range from picoseconds to hundreds of nanoseconds. It can be written as

$$\boldsymbol{\tau = \frac{1}{(k_r + k_{nr})}}$$

This is defined as the time for the intensity to drop by 1/e or to 37%. Fluorescence lifetime is useful in elucidate changes in the nanoenvironment(viscosity, pH, polarity, salvation), size and shape of molecules, molecular interactions, inter- and intramolecular distances, kinetic and dynamic rates, etc.

Fluorescence quenching: Fluorescence quenching refers to any process which decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in quenching. These included excited state reactions, molecular rearrangements, energy transfer, ground state complex formation, and collisional quenching.

The quenching rate is determined by the Stern-Volmer binding constant (K_{SV}), and in most cases it has been obtained from steady-state fluorescence quenching experiments. If only one kind of quenching mechanism is operative, the quenching can be represented by Stern-Volmer (SV) equation

$$(I_0/I) = K_{SV}[Q] + 1$$

where, I_0 and I are the fluorescence intensities before and after the addition of analytes, [Q] is the quencher concentration.

Fluorescence quenching is mainly divided into two groups *e.g.* static quenching and dynamic quenching. In the static quenching a non-emissive ground state intermediates are formed and dynamic quenching mainly deals with collisional quenching.

Fluorescence Anisotropy: Anisotropy measurements are used in the biochemical applications of fluorescence. It was based on the principle of excitation of fluorophores by polarized light. The electric vector of the excitation light is oriented parallel to the vertical axis. The intensity of emission was measured through a polarizer. When the emission polarizer is oriented parallel and perpendicular to the direction of polarized excitation, the observed intensity were $I_{\parallel}$ and $I_{\perp}$ respectively. These intensities were used to calculate fluorescence anisotropy and polarization as

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad \text{and} \quad P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Resonance Energy Trasfer: Resonance energy transfer (RET) is the transfer of energy from an energetically excited donor molecule to acceptor molecule without appearance of photon. As a

result, donor molecule will return to its ground states without emission of its luminescence, resulting luminescence quenching of donor molecule. RET occurs due to long range dipole-dipole interaction between donor and acceptor. For this reason, it is preffered to term Förster resonance energy transfer (FRET). The rate of transfer depends on:

a) The spectral overlap between the emission spectrum of the donor and the absorbance spectrum of the acceptor;

b) The quantum yield of the donor;

c) The relative orientation of the transition dipoles of the donor and acceptor;

d) The distance between the donor and acceptor.

For a donor acceptor pair, the distance at which FRET efficiency is 50%, called the Förster distance which is typically in the range of 20-60 Å. The rate of energy transfer k_T(r) is described as

$$k_T = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$

Where r is the distance between donor and acceptor, τ_D is the lifetime of the donor in absence of acceptor and R_0 is the Förster distance.

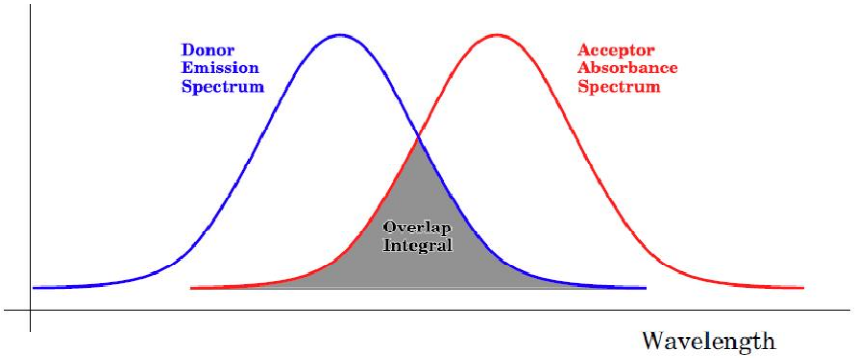

Fig. 2 This figure shows the donor emission spectrum and acceptor absorbance spectrum and overlap integral between them.

Fluorescence Measurements: Fluorescence can be measured by using steady-state and time resolved techniques.

Steady-State Fluorescence: Steady-state fluorescence is the most common measurement involving nanosecond timescale. This measurement is performed when the sample is illuminated with a continuous beam of light and is used to measure the intensity or emission spectrum.

Time resolved Fluorescence: Time-resolved fluorescence is an extension of steady state fluorescence as it requires complex and expensive instrumentation. Here, the sample is exposed to a pulse of light, where the pulse width is typically shorter than the decay time of the sample. It is used for measuring intensity decays or anisotropy decays (in nanosecond timescale).

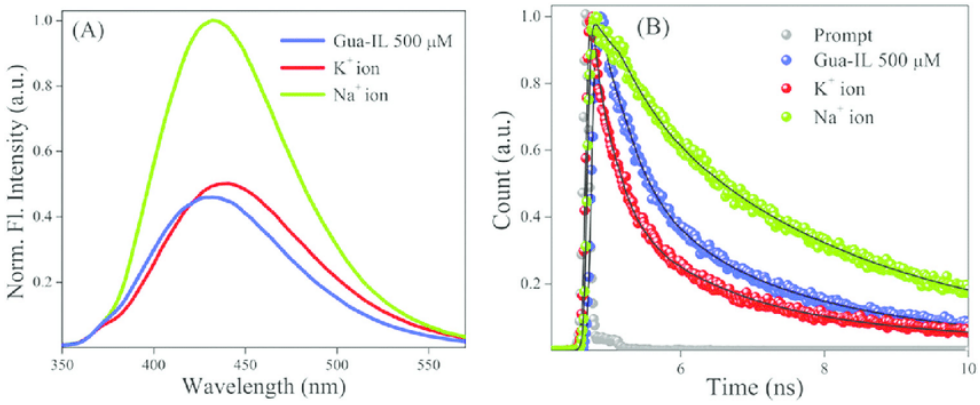

Fig. 3 (A) Steady state fluorescence and (B) time resolved fluorescence decay spectra of fluorescent modified quadruplex (B5 mM) under different conditions. The K+ ion and Na+ ion legends indicate the presence of 100 mM KCl and NaCl salt, respectively, in 10 mM tris buffer solution (pH 7.2).[Taken from Phys. Chem. Chem. Phys., 2016, 18, 29740]

Advantages of fluorescence method, which range from fluorescence spectroscopy to fluorescence microscopy, include the measurement of a number of unique experimental parameters (excitation wavelength, emission wavelength, intensity/quantum yield, fluorescence lifetime and emission anisotropy) with nanometer scale resolution and possible sensitivity down to single molecule level.[13] One particularly attractive and promising approach would involve the use of optical methods, which offer many benefits over other methods like (i) Relatively low cost (ii) Good portability (iii) High sensitivity and selectivity (iv) Short response time (v) Used in both solution and solid state.

Nanoscopic Media

The description that combines molecular level parameters and reduced to molecular level macroscopic parameters can be termed ‘nanoscopic’. The size of nanometer is of central importance in the nature and biological systems. Nanoscopic media is mainly used for

encapsulation, stabilization, solubilization and entrapment of non polar molecules i.e. hydrophobic bioactive molecules. This ability to entrap, deliver and release of water-soluble, lipid-soluble and amphiphilic materials makes them to utilize as a nano-carrier system. Nanocarriers with optimized physicochemical and biological properties are taken up by cells more easily than larger molecules, so that they can be successfully used as delivery tools for currently available bioactive compounds. This nanosized media has opened up a new potential in research and development in numerous research fields to study the individual molecules and the specific intra- and inter-molecular interactions in which they take part.[14] Micelles, reversed micelles and micro-emulsions, bilayers (vesicles, liposomes) are well-established nanoscopic media to study photochemistry of various organic molecules. These organized assemblies solubilize, concentrate and compartmentalise the substrates and catalyze various physical and chemical processes. They have extensively found bioapplications in the fields of cosmetics, food, drug delivery, imaging, medical diagnosis, tissue engineering, etc. In food industry, it is mainly used is to enrich the food products using bioactive compounds and encapsulation technology enables to protect these compounds. Various advantages of nano-sizing for drug delivery are: (i) Decrease drug resistance (ii) Decrease toxicity[15] (iii) Enhance oral bioavailability[16] (iv) Enhance rate of dissolution (v) Enhance solubility[17] (vi) Increase the stability of drug and formulation[18] (vii) Increase drug targeting ability[19-21] (viii) Increase patient compliance[18] (ix) Increase surface area (x) Reduce the dose needed.[22] Some of the nanoscopic media are discussed as follows:

Micelles: Micelles are an aggregate of amphiphilic molecules having both polar or charged groups and non polar regions in aqueous solution. In a micelle, polar or ionic heads form an outer shell in contact with water, while non polar tails are sequestered in the interior. Micelles are spherical particles (5-100 nm diameter) and have the ability to encapsulate non-polar molecules including lipids, flavorants, antimicrobials, antioxidants, vitamins, etc. Micelles help in further understanding of more complex structure like liposomes and nanococheleates. Micelles are widely used in industrial and biological fields for their ability to dissolve and move non polar substances through an aqueous medium, or to carry drugs. The functional properties of micelles are based on amphiphilic block copolymers, which assemble to form a nanosized core structure in aqueous media. The hydrophobic core region serves as a reservoir for

hydrophobic drugs, whereas the hydrophilic shell region stabilizes the hydrophobic core. In pharmaceuticals, the hydrophobic reservoir of a micelle is especially suited for the delivery or encapsulation of hydrophobic compounds.[23,24]

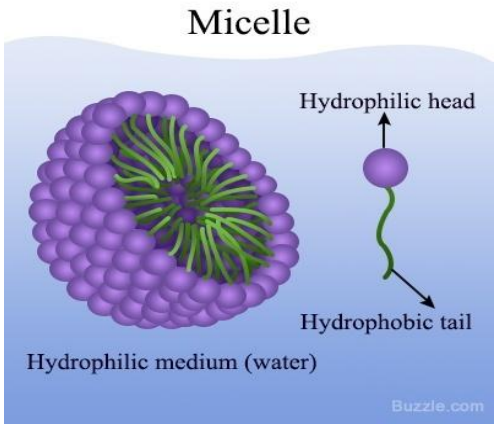

Fig. 4 Micelles

Micelles of Biosurfactants: Biosurfactants are amphiphilic compounds, structurally diverse group of surface- active molecules entirely based on renewable resources and produced through a biological process. It has several advantages over synthetic surfactants as they are less toxic, more effective, selective, environmental friendly and stable. They have the properties of reducing surface tension, stabilizing emulsions, promoting foaming and are generally non-toxic and biodegradable. They are used in food industry, enhanced oil recovery, bioremediation, environmental protection, health care and cosmetic industries, detergents, medical industries, etc. Typical examples of biosurfactants derived from animal sources include lecithin, gelatin, casein, wool fat, cholesterol and wax[25] and plant sources includes saponins, lecithin, etc.

A well-studied compound is casein. Casein is a milk protein that accounts for ~80% of milk's total protein content. It is a heterogeneous phosphoprotein that consists of four major fractions: αs1 (~44%), αs2 (~11%), β (~32%), and κ (~11%). Caseins have relatively random and flexible structures in solution; some regions contain hydrophobic residues, and others contain polar and charged residues, and thus casein tends to form micelles in solution. Casein micelles (~100–300 nm in diameter) consist of sub-micelles (10–20 nm) aggregated together. A few years back, caseins have been used as encapsulates for bioactive compounds and are widely used as emulsifiers, thickeners and gelling agents in various food products.[26-28]

Lecithin is the only truly natural low-molecular-weight surfactant found in both plants and animals. It is predominantly manufactured from soybean oilseeds due to their abundance and low cost rather than egg lecithin. Soy lecithin are widely used as emulsifiers, antioxidants, stabilizers, lubricants, wetting agents, and nutritional supplements.[29] Lecithin in solutions forms micelles and liposomes, self-organizes into liquid-crystalline structures, disperses and stabilizes dispersions.

Gelatin is a relatively poor protein surfactant, but its emulsifying properties can be improved by enzyme-catalyzed attachment of hydrophobic side chains[26] or by combination with other surface-active agents. It is a product obtained through the partial hydrolysis of collagen with dilute acid or base. The main sources of commercial gelatin are bovine skin and bones and pigskin. Gelatin is a high-molecular-weight polymer and had been used as a stabilizer, thickener, and texturizer in food and non-food applications.[30]

Saponins are amphiphilic molecules in which sugars are linked to either a sterol or a triterpene non-polar group. Soy protein is one of the most important plant derived protein surfactants. They can be isolated from different plant parts and most significant sources includes legumes: soybeans, chickpeas, mung beans, peanuts, broad beans, kidney beans and lentils; the saponin content in soybeans is 5–6%.[25] Saponins have emulsifying and foaming properties, pharmacological and medicinal properties, and antimicrobial and insecticidal activity and are used in beverages, confectionery, cosmetics and pharmaceutical products.[31,32]

Reverse Micelles: Reverse micelles are nanometer-sized (1-10 nm) water droplets dispersed in organic media obtained by the action of surfactants. Surfactant molecules organize with the polar part to the inner side able to solubilize water and the apolar part in contact with the organic solvent. Water and hydrophilic molecules such as biological active compounds can be solubilised inside the core of the reverse micelles. It plays major role for selective solubilisation of biomolecules which provides a logical aqueous environment protecting the bioactivities of the biomolecules.[33]

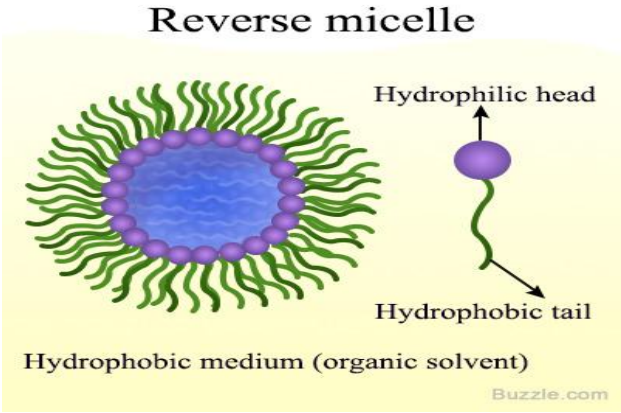

Fig. 5 Reverse micelles

Microemulsions: Microemulsions are are thermodynamically stable isotropic mixtures of oil, water and surfactant; usually with a co-surfactant. The oil was composed of a mixture of different hydrocarbons and olefins. Microemulsions was considered as small-scale versions of emulsions, i.e either of oil-in-water (o/w) or of water-in-oil (w/o), with a size range in the order of 5−50 nm in drop radius. Variable reaction media depends on the (w/o) or the (o/w) ratios and have been used to study synthesis of organic compounds,[34-36] organic reactivity[37] such as keratin cystine reactivity,[38] etc. It overcome the solubility and incompatibility problems of the reactants and used as reaction media for the synthesis of surfactants,[39-41] organic conversions as well as for analytical chemistry.[42,43]

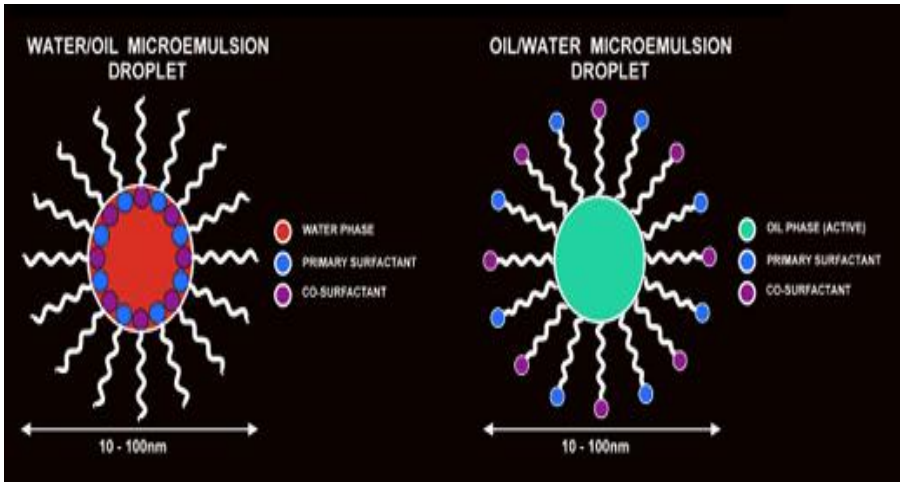

Fig. 6 Microemulsion

Liposomes: Liposomes are spherical particles composed of one or more lipid and or phospholipid bilayers and contain spacing between the bilayers. Liposomes have been the first to be investigated as drug carriers. They are nano-sized colloidal carriers, usually with 80–300 nm size range.[44] Liposomes represent versatile and advanced nano-delivery systems for a wide range of biologically active systems. It helps in encapsulation, protection and controlled release of bio actives which have used as a subject of research in now a days. It reduces the toxicity of encapsulating agent and increased stability via encapsulation. It can trap both hydrophobic and hydrophilic compounds.

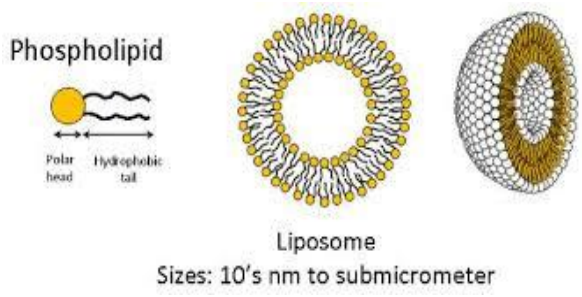

Fig. 7 Liposome

Vesicles: Vesicles is a small structure of nanometer size within a cell, consisting of fluid enclosed by a lipid bilayer of phospholipids. These vesicles store, transport, and digest waste and products from the cell. Vesicles are important since they play a role in metabolism, transport, enzyme storage, and are chemical reaction chambers and are used as drug delivery agents, model components for cell membranes and cationic softeners in detergency.

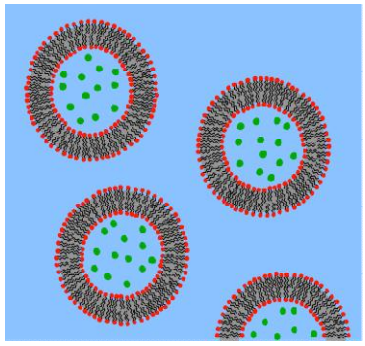

Fig. 8 Vesicles

Nanocochleates: Cochleates are small-sized and very stable delivery system with a multilayered structure consisting of a large, continuous, solid lipid bilayer sheet rolled up into a spiral. They

can be applied to encapsulate many bioactive materials such as compounds with poor water solubility, peptide, protein, drugs and large hydrophilic molecules.[45] Nano-cochleates have a great potential to deliver bioactive agents due to its stability and nano size.

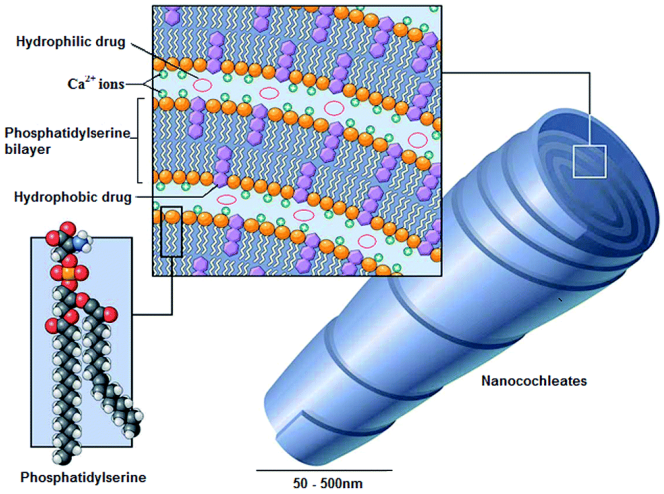

Fig. 9 Schematic illustration of drug delivery via nanocochleate technology. [Adapted from Food Eng Rev., 2012, **4**, 114]

Dendrimers: A dendrimer is a synthetic polymeric macromolecule of upto 10 nanometer dimensions, composed of multiple highly branched monomers that emerge radially from the central core. Dendrimers which have regular, highly branched 3-dimensional structure can be applied as sensors, catalysts, delivery of drugs, immunoassay, MRI contrast agent and in gene therapy.[46] Dendrimers are mainly used as delivery system as they are nontoxic, nonimmunogenec and biodegradabe.[47] There are a few ways of connecting biologically active compounds to dendrimers. The drug may been capsulated in the internal structure of dendrimers[48] or it can be chemically attached or physically adsorbed on dendrimers surface.[49]

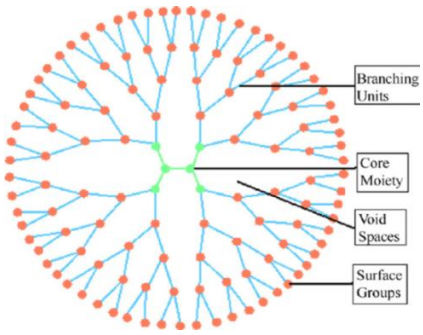

Fig. 10 Dendrimer

Bioactive Compounds in Nanoscopic Media:

Hanif et al. studied the solubilization and behaviour of bioactive compound Benzothiazole (BNZ) by the micellar media of an anionic surfactant, sodium dodecyl sulphate(SDS) and a cationic surfactant, cetyltrimethylammonium bromide(CTAB).[50] The spectroscopic results demonstrate high degree of solubilization and strong host guest relationship between BNZ and micelles and here BNZ increases the CMC of both surfactants. The thermodynamic parameters suggest that the process of solubilization of BNZ in the micellar media is entropy driven and spontaneous. The change in CMC of surfactant in the presence of organic additive, partition coefficient, binding constant, free energies of partition and binding provides a useful way to study solubilization of bioactive compounds by micelle. Earlier, he also studied the solubilization of Chloroquine Diphosphate[51], Quinacrine2HCl[52] and Pefloxacinmesylate[53] by micellar media.

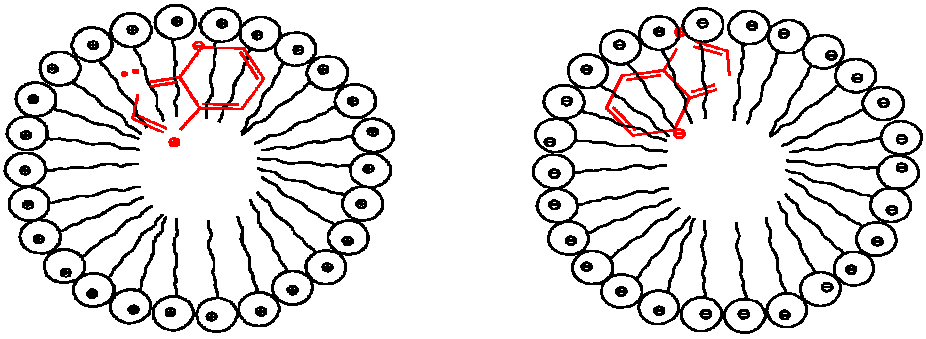

Fig.11 (a) Partitioning of BNZ in a SDS micelle, (b) Partitioning of BNP in a CTAB micelle [Taken from Journal of Molecular Liquids, 2015, **211**, 7]

Hanif et al. also reported the partitioning of benzothiophene (BNP) between the aqueous phase and micellar media of an anionic surfactant, sodium dodecyl sulphate and a cationic surfactant, cetyl trimethyl ammonium bromide (CTAB).[54] The experimental results reveal that in the premicellar region, the molecules of BNP get attached with monomeric surfactant molecules, while in the post-micellar region; they are accommodated within the micelle. The bathochromic shift is observed in the UV/visible spectra of BNP in the presence of both surfactants and the water/micelle partition coefficient (Kx) show that the solubilization of BNP is

more spontaneous and takes place to a greater extent in micellar media of SDS than in CTAB.

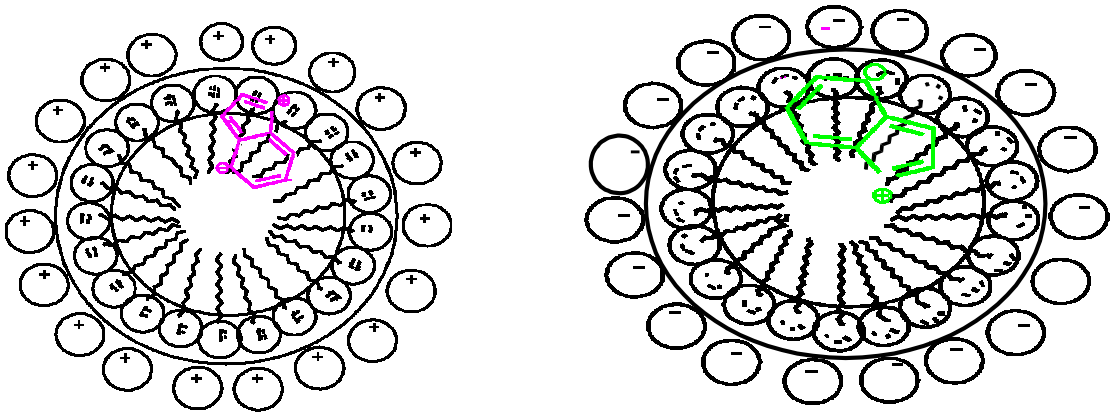

Fig. 12 (a) Partitioning of BNP in an CTAB micelle, **(b)** Partitioning of BNP in a SDS micelle [Taken from J Surfact Deterg., 2016, **19**, 1033]

Begum et al. studied steady-state and time resolved fluorescence spectroscopy of naturally occurring carbazole alkaloid murrayanine in homogeneous alcohol solvents as well as in aqueous micelles.[55] It was found that fluorescence peak position and intensity are extremely sensitive to the nature of the solvent medium so that murrayanine could be used as an excellent fluorescence probe for the micellization of Pluronic F68, an amphiphilic copolymer with possible application in drug-delivery[56] and murrayanine fluorescence has been further utilized to determine the critical micellization concentration of Pluronic F68.

Paul et al. studied the interaction of a promising cancer cell photosensitizer harmane (HM) with micellar assemblies of varying surface charges.[57] The binding interaction of the drug with the micelles is found to be governed by a conjugate effect of polarity and rigidity of the micellar microheterogeneous environments. The results of steady-state and time-resolved fluorometric studies reveal the differential modulation of the prototropic equilibrium of the drug (HM) in micellar environments of varying surface charges. The results of micropolarity measurement at the drug interaction site within the micelles combined with the results of fluorescence quenching experiments delineate the probable location of the drug in the Stern layer of the cationic and neutral micellar systems whereas in the anionic micellar system the drug is probably localized in the micellar interfacial region.

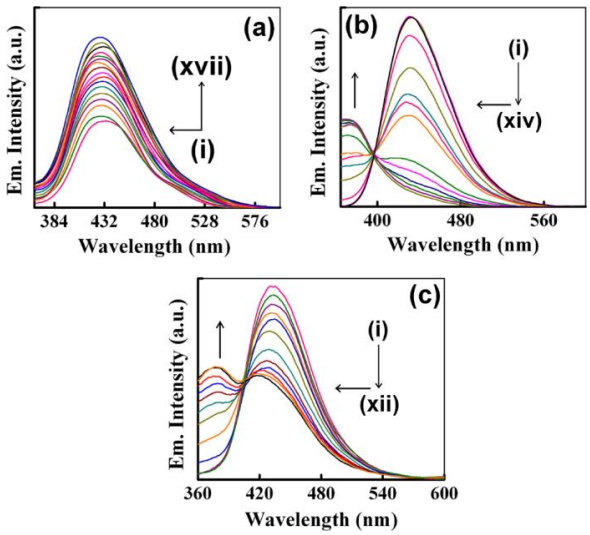

Fig.13 Representative emission spectral profile of HM (λ_{ex} = 350 nm) in aqueous buffer medium in the presence of increasing concentrations of (a) SDS, (b) CTAB, and (c) TX-100. In panel (a) curves (i) → (xvii) correspond to [SDS] = 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mM. In panel (b) curves (i) → (xiv) correspond to [CTAB] = 0.0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, 5.0, and 7.0 mM. In panel (c) curves (i)→ (xii) correspond to [TX-100] = 0.0, 0.022, 0.044, 0.088, 0.13, 0.18, 0.22, 0.26, 0.31, 0.35, 0.40, and 0.44 mM. [Taken from J. Phys. Chem. B, 2012, **116**, 9704]

Mallick et al. reported the photophysical properties of a bioactive molecule 3-acetyl-4-oxo-6,7-dihydro-12*H*-indolo[2,3-*a*]quinolizine (AODIQ) in mono-dispersed biomimick-ing nanocavities formed by sodium bis(2-ethylhexyl)sulfosuccinate (AOT) in heptane using steady-state and picoseconds time resolved fluorescence and fluorescence anisotropy.[58]

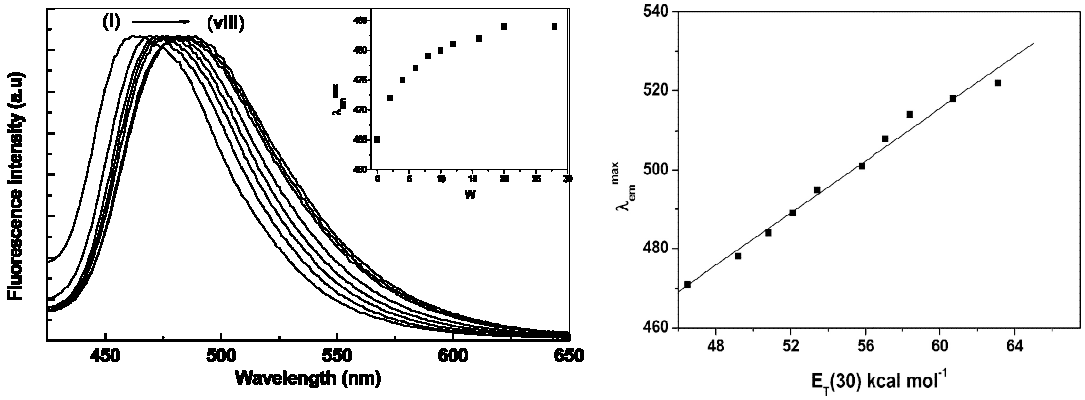

Fig.14 (a) Normalized fluorescence spectra of AODIQ in AOT/*n*- heptane reverse micelles as a function of *W* (λexc = 420 nm). Curves i-viii correspond to *W* = 0, 2, 4, 6, 10, 16, 20, and 28, respectively. The inset shows the variation of fluorescence maximum as a function of *W*, **(b)** Variation of emission maximum of AODIQ in the dioxane-water mixture. [Adapted from J. Phys. Chem. B**,** 2005**, 109***,* 14675]

Mallick et al. also reported the interaction of a biologically active molecule 3-acetyl-4-oxo-6,7-dihydro-12H indolo-[2,3-a]quinolizine (AODIQ) with model transport proteins, bovine

serum albumin (BSA) and human serum albumin(HSA) using steady state and picosecond time-resolved fluorescence and fluorescence anisotropy.[59] The results shows that the polarity dependent intramolecular charge transfer(ICT) process is responsible for the remarkable sensitivity of this biological fluorophore to the protein environment and the CT fluorescence exhibits appreciable hypsochromic shift along with an enhancement in the fluorescence yield, fluorescence anisotropy (*r*) and fluorescence lifetime upon binding with the proteins. The reduction in the rate of ICT within the hydrophobic interior of albumins leads to an increase in the fluorescence yield and lifetime. Marked increase in the fluorescence anisotropy indicates that the probe molecule is located in a motionally constrained environment within the protein

Gupta et al. developed a stable clove oil-in-water microemulsion with droplet dimensions of 9–20 nm that could favorably encapsulate the oil-soluble quinonoid compound, diospyrin which is a highly lipophilic bioactive compound and examined as a delivery system for *in vivo* application.[60] In 2001, Moreno et al. reported a o/w microemulsion for delivery of another lipophilic drug, amphotericin B used in the treatment of several types of leishmaniasis.

Kumari and co-authors achieved a controlled release of quercetin by its encapsulation into poly-D,L-lactide (PLA) nanoparticles through solvent evaporation technique.[61] Quercetin was also encapsulated in Pluronic F127 through supercritical anti-solvent method by Fraile and co-authors, enabling an improved dissolution behaviour of the compound in simulated physiological fluids. Althans and co-authors have demonstrated that by using hyperbranched polymers and hydrogels as quercetin delivery systems it was possible to increase its water solubility and to enhance the chemical stabilization of the flavonoid, respectively. Calabrò et al. used cyclodextrins (CD), such as α-CD, β-CD or HP-β-CD for the encapsulation of quercetin using the freeze-drying or co-evaporation method. Barras and co-authors used lipids to encapsulate quercetin, being able to increase its apparent aqueous solubility by a factor of 100. Quercetin-loaded liposomes have also been produced and are reported in the literature by Priprem et al. in 2008. In 2012, De Paz and co-authors developed a novel method for the encapsulation of hydrophobic compounds, based on the production and processing of ethyl acetate-water emulsions at high pressure and temperature. The authors achieved stable aqueous suspensions of β-carotene with micellar particle sizes down to 400 nm and encapsulation efficiencies up to 80%. Cocero and co-workers reported the formulation of a type of flavonoid

antioxidant quercetin, using three distinct emulsifiers, namely n-octenylsuccinateanhydride starch, soybean lecithin and barley β-glucan, through pressurized ethyl acetate-in-water emulsions. Results shows that lecithin yielded the best results, with encapsulation efficiencies up to near 76%, increasing its solubility and particle sizes in the sub-micrometer range. The addition of glycerol as co-solvent can increase the colloidal stability of the suspension and the encapsulation efficiency of the flavonoid.

Henriques and co-workers reviewed on the use of cyclodextrins as encapsulating agents for bioactive plant molecules as cyclodextrins are inexpensive, friendly to human, capable of improving the biological, chemical and physical properties of bioactive molecules and protect them from elevated temperatures, pH values, light or the moisture induced degradations phenomena which serve to increase their bioavailability.[62] It was shown that CD derivatives are the better choice to achieve an efficient complexation between CD and plant phenolics.

Proposed plan of work:

To date many works on synthesis or extraction of bioactive compounds using solvents like ethanol, chloroform, dichloromethane, acetone, etc. have been reported but works on the synthesis of these compounds in nanoscopic media are rare. Generally bioactive compounds are synthesized using organic solvents as organic reagents are soluble in it but there is a scope to synthesize these compounds in nanoscopic media from organic reagents. Nanoscopic media is mainly used for encapsulation, stabilization and entrapment of hydrophobic bioactive molecules as well as to increase the solubility and stability of the encapsulated compound. The spectroscopic and photophysical data of this molecular systems in nanoscopic media is most promising for a better understanding the probable location of the compound in this environment, its intetraction with a particular biological targets like proteins, drugs, etc.

I propose to synthesize bioactive compounds from organic reagents in nanoscopic media (using surfactants or biosurfactants in aqueous medium as it is capable of solubilizing hydrophobic molecules i.e. organic reagents) or extract it from renewal sources (from plant sources or fruit peels, etc) , to characterize them, to determine their structures, and to investigate luminescence properties such as fluorescence quantum yield, binding constant, fluorescence lifetime, fluorescence anisotropy etc. and related application towards heath, food and beverages, medical, nutritional, pharmaceutical industries, etc.

Objectives:

1. To synthesize bioactive compounds from organic reactions in nanoscopic media or to extract it from natural resources
2. To determine the structure of the compound by using NMR, IR, etc.
3. To characterize them using TGA, IR, UV-Vis, etc.
4. Detailed spectroscopic study such as Forster resonance energy transfer, electron transfer, fluorescence anisotropy etc. using steady state and time resolved fluorescence spectroscopy of the bioactive compounds
5. Finding the application of the studied compound towards heath, food and beverages, medical, nutritional, pharmaceutical industries, etc.

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